ELISA PROTOCOL
ANTIGEN SPECIFIC IgG_1/IgG_{2a}

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STEP	*mL/WELL*	*DILUTION*	*BUFFER*	*INCUBATION*
Coat with Diluted Antigen – Hp, Tm, or Nb Extract	50	5µg/mL	BBS	2 hrs. RT Overnight 4°C
Wash Plates 3 times	300		ddH_20	
Block the Plate	50		PCS	30 min. RT
Wash Plates 3 times	300		ddH_20	
Apply Samples Samples - Start with 1:20 and do 1:4 serial dilutions	50		BBS + Tween	2 hrs. RT Overnight 4°C
Wash Plates 3 times	300		ddH_20	
Incubate with Wash Buffer	50		BBS + Tween	30 min. RT
Wash Plates 3 times	300		ddH_20	
Incubate with Detection Antibody - α-mouse IgG-AP IgG_1 - Southern Biotechnology Associates Cat # 1070-04 IgG_{2a} - Southern Biotechnology Associates Cat # 1080-04	50	1:2000	BBS + Tween	30 min. RT
Wash Plates 3 times	300		ddH_20	
Incubate with Wash Buffer	50		BBS + Tween	30 min. RT
Wash Plates 3 times	300		ddH_20	
Add Substrate and Develop - MUP MUP - Sigma Cat # M-8883	50	5mg/100mL	MUP Buffer	Until Highest Standard Reaches 2800 F.U.
Detect Fluorescence on ELISA Plate Reader				

ELISA protocol developed using Immulon IV plates from Dynex, Cat #3855.

Manufacturer Phone Numbers:

1. Southern Biotechnology Associates, Inc. – 205.945.1774
2. Sigma – 800.325.3010
3. Dynex – 703.631.7800

NOTE: Protocol does not work with IgG_{2a}-Tm.

ANTIGEN-SPECIFIC IgG_1/IgG_{2a} ELISA BUFFERS

1. Borate Buffered Saline (BBS)

Begin with 5L ddH_20 in 6L Ehrlenmeyer flask.
Add the following slowly with stirring:
H_3BO_3 : 61.8 g
NaCl : 43.8 g
Adjust pH to 8.5 with 1N NaOH.
Bring volume to 6L with ddH_20.
Store at Room Temperature (RT).

2. BBS + Tween

Add 10mL of 1% Tween-20 stock to 500mL BBS.
Store at Room Temperature.

3. Protein Carrier Solution (PCS)

Add 5mL Fetal Bovine Serum to 500 mL BBS
Add 5mL 10% Sodium Azide.
Store at 4°C.

4. MUP Buffer

Begin with 5L ddH_20 in 6L Ehrlenmeyer flask.
Add 25.2g $NaHCO_3$ and 1.21g $MgCl_2 \bullet 6H_20$
Adjust pH to 9.7 with 1N NaOH.
Bring volume to 6L with ddH_20.
Store at Room Temperature (RT).